WO 00/32772

- 70 -

```
WE CLAIM:
```

30

```
A protein selected from the group consisting of:
            1.
                  a) NGE;
                 b) NGE [5E];
 5
                  c) MR-NGE;
                 d) MR-NGE-88E;
                 e) MR-NGE-88K;
                  f) MR-NGE-88P;
10
                 g) MR-NGE-88S;
                 h) MR-NGE [4E];
                 i) MR-NGE[5E];
                 j) MR-NGE[5K];
                 k) MR-NGE[W5E]; and
15
                 1) MR-NGE [W5K].
                A protein selected from the group consisting of:
                 a) NGE-166\Delta;
                 b) NGE [5E] -166\Delta;
20
                 c) MR-NGE-166\Delta;
                 d) MR-NGE-88E-166\Delta;
                 e) MR-NGE-88K-166\Delta;
                 f) MR-NGE-88P-166\Delta;
                 g) MR-NGE-88S-166\Delta;
25
                 h) MR-NGE [4E] -166\Delta;
                 i) MR-NGE [5E] -166\Delta;
                 j) MR-NGE [5K] -166\Delta;
                 k) MR-NGE [W5E] -166\Delta; and
                 1) MR-NGE [W5K] -166\Delta.
```

3. The protein of Claim 2, wherein the protein is MR-NGE-166 Δ .

WO 00/32772

30

- 4. The protein of Claim 2, wherein the protein is MR-NGE[W5K]-166 Δ .
- 5 5. The protein of Claim 2, wherein the protein is MR-NGE[W5E]-166 Δ .
- 6. An erythropoietic compound having a protein portion and a polymer portion, wherein the protein portion is selected from the group consisting of: non-glycosylated human erythropoietin and non-glycosylated erythropoietin analogs and wherein the polymer portion consists of 1 to 5 polymer chains of the formula:

15
$$[R-O-(CH_2CH_2-O)_{X}-(CH_2)_{Y}-NH]$$

wherein R is H or C₁ to C₄ alkyl, X is a number from about 70 to about 1200, and Y is a number from 1 to 4; and the polymer chain is covalently bonded to the protein portion by a secondary amine bond.

- 7. The erythropoietic compound of Claim 6 wherein X is a number from about 225 to about 1200.
- 8. The erythropoietic compound of Claim 7 wherein X is a number from about 340 to about 1200.
 - 9. The erythropoietic compound of Claim 8 wherein X is a number from about 450 to about 1200.
 - 10. The erythropoietic compound of Claim 9 wherein X is a number from about 450 to about 700.

WO 00/32772 PCT/US99/27801

- 72 -

- 11. The erythropoietic compound of any one of Claims 6 through 10 wherein the protein portion is a non-glycosylated erythropoietin analog and the polymer portion is bound to the protein portion at the N-terminus of the protein.
- 12. The erythropoietic compound of any one of Claims 6 through 10 wherein the protein portion is a protein of Claim 1.

10

5

- 13. The erythropoietic compound of any one of Claims 6 through 10 wherein the protein portion is a protein of Claim 2.
- 14. A protein which is the product of the expression in a host cell of an exogenous DNA sequence comprising a DNA sequence encoding at least one of the proteins of Claim 1 or 2.
- 20 15. The erythropoietic compound of any one of Claims 6 through 10 made by a process comprising the steps of:
 - a) adding a polyethylene glycol-aldehyde polymer to a solution of non-glycosylated erythropoietic protein under conditions that permit the formation of an imine bond between an amino group of the protein and the aldehyde group of the polymer; and
 - b) adding a reducing agent to reduce the imine bond to a secondary amine bond.

30

25

16. An isolated nucleic acid sequence, comprising a polynucleotide encoding a protein of Claim 1 or Claim 2.

WO 00/32772 PCT/US99/27801

- 73 -

- 17. An isolated nucleic acid sequence, comprising a polynucleotide which is SEQ ID NO:2.
- 5 18. A vector comprising a nucleic acid sequence according to Claim 16 or 17.
 - 19. A host cell comprising the vector of Claim 18.
- 10 20. A host cell expressing at least one protein of Claim 1 or Claim 2.
 - 21. The host cell of Claim 19 or 20 wherein said host cell is E. coli.
 - 22. A transgenic or chimeric non-human animal, comprising at least one host cell according to Claim 20.
- 23. A process for producing a protein comprising the steps of transcribing and translating the isolated nucleic acids of Claims 16 or 17 under conditions that the protein is expressed in detectable amounts.
- 24. A process for preparing polymer-derivatized, non-25 glycosylated erythropoietic compounds, comprising the steps of:
 - a) adding a polyethylene glycol-aldehyde polymer to a solution of non-glycosylated erythropoietic protein under conditions that permit the formation of an imine bond between an amino group of the protein and the aldehyde group of the polymer; and
 - b) adding a reducing agent to reduce the imine bond to a secondary amine bond.

30

15

WO 00/32772 PCT/US99/27801

- 74 -

25. The process of Claim 24, wherein the aldehyde polymer and the protein in the solution are present at a ratio of 0.08 to 24 on a mole polymer per mole protein basis.

5

- 26. The process of Claim 25, wherein the ratio of aldehyde polymer to protein is 1 to 10.
- 27. The process of any one of Claims 24 through 26, wherein the solution is buffered and the added reducing agent is selected from the group consisting of sodium cyanoborohydride and sodium borohydride.
- 28. The process of Claim 27, wherein the solution is borate-phosphate buffered with a pH between 7 and 9 and said reducing agent is sodium cyanoborohydride.
- 29. A method for increasing the hematocrit levels in a 20 mammal comprising the administration of a therapeutically effective amount of an erythropoietic compound of any one of Claims 6 through 10.
- 30. The use of an erythropoietic compound as claimed in any one of Claims 6 through 10 for the manufacture of a medicament for the treatment of patients with insufficient hematocrit levels.
- 31. A pharmaceutical formulation adapted for the treatment of patients with insufficient hematocrit levels comprising an erythropoietic compound of Claims 6 through 10.
- 32. An erythropoeitic compound as hereinbefore 35 described with reference to any one of the Examples.